UV-screening and springtime recovery of photosynthetic capacity in leaves of Vaccinium vitis-idaea above and below the snow pack

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Title: UV-screening and springtime recovery of photosynthetic capacity in leaves of *Vaccinium vitis-idaea* above and below the snow pack

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Declaration of Authorship: TMR SMH conceived & designed the experiments. TS, SN, SMH, MP TMR, did the majority of the field work and sampling, TS, MP, AS, PJA TMR analysed the data, JAPC JA provided site-specific help, and access to meteorological data integral to the experiment, TS, PJA, TMR wrote the manuscript, and all authors provided editorial input.

Abstract (limit 250/250 Words)

Evergreen plants in boreal biomes undergo seasonal hardening and dehardening adjusting their photosynthetic capacity and photoprotection; acclimating to seasonal changes in temperature and irradiance. Leaf epidermal ultraviolet (UV)-screening by flavonols responds to solar radiation, perceived in part through increased ultraviolet-B(UV-B) radiation, and is a candidate trait to provide cross-photoprotection. At Hyytiälä Forestry Station, central Finland, we examined whether the accumulation of flavonols was higher in leaves of *Vaccinium vitis-idaea* L. growing above the snowpack compared with those below the snowpack. We found that leaves exposed to colder temperatures and higher solar radiation towards the top of hummocks suffered greater photoinhibition than those at the base of hummocks. Epidermal UV-screening was highest in upper-hummock leaves, particularly during winter when lower leaves were beneath the snowpack. There was also a negative relationship between indices of flavonols and anthocyanins across all leaves suggesting fine-tuning of flavonoid composition for screening vs. antioxidant activity in response to temperature and irradiance. However, the positive correlation between the maximum quantum yield of photosystem II photochemistry (F_v/F_m) and flavonol accumulation in upper hummock leaves did not confer on them any greater cross-protection than would be expected, from the relationship of F_v/F_m with temperature and irradiance during dehardening present among leaves throughout the hummocks. Irrespective of timing of snow-melt, photosynthesis fully recovered in all leaves, suggesting that *V. vitis-idaea* has the potential to exploit the continuing trend for longer growing seasons in central Finland without incurring significant impairment from reduced duration of snow cover.

41 **Graphical Abstract**: Schematic representing the changes in leaf level processes moderated by the
42 snowpack, sunlight and temperature after different heights on hummocks of *V. vitis-idaea* during
43 spring dehardening

44

45 **Keywords** *(7 max)*: spring dehardening; flavonoids; anthocyanins; understorey; spectral quality;
46 photoprotection; Arctic browning

47

1. Introduction

An expected outcome of ongoing human-induced climate change is a shift in seasonal precipitation and temperature affecting growing season length (Harsch and HilleRisLambers 2016; IPCC 2014). This has contributed to a reduction in annual snow-cover in the Arctic by as much as 5 days each decade since 1979 (Derksen et al. 2016). At high northern latitudes, less of the winter precipitation is expected to fall as snow and more as rain (Mellander et al. 2007; Rasmus et al. 2004) and temperatures favourable for growth are forecast to occur earlier in the spring (Henry 2008; Jylhä et al. 2008). In Finland, mean annual temperature is expected to increase by 4-11°C and precipitation (and hence cloudiness) by 10-60 % by the end of 21st century, with most increases occurring during the winter (Finland's Seventh National Communication under the United Nations Framework Convention on Climate Change, 2017). The benefit of this change for plant growth and photosynthesis is uncertain because warmer mean temperatures together with reduced precipitation as snow may lead a smaller snow pack that melts earlier (Kreyling et al. 2012). The absence of snow cover will imply greater temperature fluctuations close to the ground, exposing plants to colder minimum, and warmer maximum, temperatures when they are starting to deharden at the end of winter (Blume-Werry et al. 2016; Preece and Phoenix, 2013).

Plants possess several photoprotective mechanisms which mitigate abiotic stresses under harsh conditions: (1) when reaction centres are open photochemical quenching ($_qP$) entails activation of the enzymes and metabolites involved in charge separation to produce energy via photosynthesis; (2) excess light energy, causing reaction centres to close, is dissipated through xanthophyll-cycle pigments (carotenoids) zeaxanthin and antheraxanthin in a process regulated by the pH the thylakoid lumen (Adams et al. 2004; Murchie and Lawson 2013); (3) additionally antioxidants such as phenolic compounds are accumulated to quench reactive oxygen species (ROS) (Agati et al. 2013; Hideg et al. 2013; Salter et al. 2017). It is reasonable to assume that the pressure on these mechanisms is increased when a plant is subject to both fluctuating temperatures and irradiances above the snowpack rather than the more stable conditions offered under snow (Loik et al. 2004; Preece and

Phoenix, 2013). Low temperatures dampen physiological processes meaning that even moderate irradiances cause photoinhibition through photoinactivation via the Mn_4CaO_5 cluster or inhibition of the photosystem II (PS_{II}) electron acceptors (Neuner et al. 1999; Saarinen et al. 2011). Sunlight can penetrate the snowpack, where temperature fluctuations are small, potentially allowing some photosynthesis at low irradiances (Saarinen et al. 2016). Some studies have found the relative transmittance of different regions of the spectrum by snow to be quite even (Gorton and Vogelmann, 2003) while others have reported spectrally-differential attenuation through the snowpack (Curl Jr et al. 1972; Richardson and Salisbury, 1977). Although solar irradiance, particularly ultraviolet-B (UV-B: 280-315 nm) radiation, is low in winter, the ratio of ultraviolet (UV-B plus UV-A: 280-400 nm) to photosynthetically active radiation (PAR: 400-700 nm) in forest understorey shade is enriched (Jansen et al. 2017; Hartikainen et al. 2018).

Flavonoids such as flavonols and anthocyanins are the most-common class of phenolic compounds produced in response to UV-radiation and blue light (Searles et al. 2001; Sullivan et al. 2007; Taulavuori et al. 2016). Flavonols accumulate in the leaf adaxial epidermis absorbing UV-radiation so screening the photosynthetic apparatus in the mesophyll below (Barnes et al. 2017; Caldwell et al. 1983; Nybakken et al. 2004). Although their absorption spectrum is predominately in the UV region, it has been proposed that flavonoids provide cross-protection to high solar irradiance in general (Jansen et al. 2017), and a positive correlation is sometimes reported between flavonoid accumulation and the yield of PS_{II} (Robson et al. 2014; Wargent et al. 2011). This relationship may be attributable to protection against PS_{II} photodamage or enhanced ROS scavenging conferred by flavonoids (reviewed by Takahashi and Badger, 2011). The accumulation of flavonoids in response to UV-radiation is moderated by temperature, being less pronounced in warm than in cool temperatures (Bilger et al. 2007; Coffey et al. 2017; Petridis et al. 2016) where flavonoids can be important in mitigating against oxidative stress (Lütz et al. 2005) and can increase frost hardiness (Chalker-Scott and Scott 2004; Shulze et al. 2016). Hence, the accumulation of flavonoids could provide a feasible

99 strategy whereby evergreen plants ameliorate photoinhibition, imposed by increasing irradiance and
100 temperature fluctuations, as they deharden over the spring.

101 Boreal evergreen and wintergreen plants adjust light harvesting complexes to optimise
102 quenching processes at the expense of the light harvesting and photosynthetic capacity of their leaves
103 during winter when light and temperature conditions are unfavourable (Adams et al. 2004).
104 Subsequently, they undergo a recovery period in preparation for more favourable growth conditions
105 in late spring and summer (Muller et al. 2005). During this period of dehardening, full functionality
106 returns to the photosynthetic apparatus of leaves and this can be measured using chlorophyll
107 fluorescence as an increase in the yield of PS_{II} (Murchie and Lawton, 2013; Saarinen et al. 2011).
108 Monitoring leaf-level chlorophyll fluorescence of evergreen trees (Ensminger et al. 2004; Porcar-
109 Castell et al. 2008) and field-layer plants (Starr and Oberbauer, 2003) through spring in the boreal
110 forest, has revealed that recovery of F_v/F_m is modulated by temperature and light.

111 Here, we aimed to compare leaf epidermal flavonoids (flavonols and anthocyanins), chlorophyll
112 content and the PS_{II} yield at different positions on hummocks relative to the snowpack, through a
113 seasonal time-course of measurements of evergreen-shrub *Vaccinium vitis-idaea* L. During the spring
114 dehardening period, the over-wintering leaves of this species are adapted to take advantage of
115 favourable early-spring conditions by photosynthesizing when still under the snowpack (Lundell et al.
116 2008; Saarinen et al. 2011; Starr and Oberbauer, 2003). Our hypothesis was that leaves at the top of
117 the hummock above the snowpack during winter and early spring would suffer greater photoinhibition
118 because they encounter fluctuating temperatures and higher irradiance than those at the base of
119 hummocks receiving lower irradiance, of both PAR and UV radiation, and more stable temperatures
120 remaining closer to 0°C under the snow. However, we also expected higher accumulation of
121 epidermal UV-absorbing compounds (flavonols and anthocyanins) in leaves above the snow, and for
122 this to partially ameliorate the photoinhibition they suffered relative to those at equivalent
123 temperatures beneath the snowpack.

2. Methods

2.1. Study site and species

The study was performed under an open *Pinus sylvestris* L. canopy, basal area 23 m^2ha^{-1}, on level ground at Hyytiälä Forestry Station of the University of Helsinki in central Finland (61.84741°N 24.29477°E 181 m asl). The hummock-forming evergreen shrub *V. vitis-idaea* covers vast areas of peatland and boreal forest at high latitudes in the Northern Hemisphere (Karlsson, 1985; Lundell et al. 2008). At our site, the understorey comprises almost exclusively *V. vitis-idaea* in hummocks of up to 75 cm in height intermingled with *Vaccinium myrtillus* L. Four hummocks, positioned at least a metre from the nearest pine tree, were selected at random within a 10-by-10-m area of understorey. These hummocks were divided vertically into three height categories on 14-02-2017: the "upper" hummock being the uppermost shoots, most exposed to the wind and sun, the "middle" hummock being the outer shoots on the south side of the hummock, somewhat sheltered from the wind but just below the snow pack through the winter, and the "lower" hummock being shoots at the very base of the hummock on the south side, below the snow pack during the whole winter and early spring.

The snow depth relative to the lower, middle and upper hummocks, and the leaf phenology of the *Vaccinium* species, was monitored throughout the study concomitantly with leaf traits, and these data were compared against a continuous monitoring by Phenocam time-lapse images nearby in the stand (Kolari et al. 2017). By 19-06-2017, plants of both *V. vitis-idaea* and *V. myrtillus* produced new leaves. From this date onwards, until our final measurement date, 08-11-2017, a set of measurements was also made on these new *V. vitis-idaea* leaves (unfurling in June 2017) in same way as those on leaves produced in June 2016. This allowed us to obtain data for leaves from the time of their unfurling through their first and second years of growth. To allow this continuum of leaf age to be visualised in the figures we have combined these two set of leaf traits in a single time series; with 2017 leaves plotted from their unfurling until 08-11-2017 (day 312 of the year, leaf age 152 days) and 2016 leaves from 14-02-2017 onwards (day 45 of the year, leaf age 250 days).

2.2. Characterisation of the forest canopy by hemispherical photograph

Plant area index (PAI)(m^2m^{-2}), was estimated at each of the four hummocks by taking hemispherical photographs (at 40 cm height from the ground) through a Sigma 4.5mm f2.8 EX DC HSM circular Fisheye lens (Sigma Corporation of America, Ronkonkoma, NY) on a Nikon D7000 camera (Nikon corporation, Tokyo, Japan) levelled on a tripod. Measurements were done on a completely overcast day in mid-July (20-07-2017) to ensure homogeneous grey sky and a good contrast between the sky and the canopy (Fig S1). All photos were analysed following the procedure detailed in Hartikainen et al. (2018).

2.3. Measurement of spectral irradiance at the study site

The solar spectral irradiance was measured close to solar noon, directly to the south of each of the four hummocks on 07-03-2017 and 19-06-2017 in sunflecks and canopy shade on completely clear-sky days. These data were compared with corresponding measurements in an open field next to the forest stand earlier on the same days. Spectral irradiance recordings were made using a Maya 2000 Pro (Ocean Optics, Dunedin, FL, USA) array spectrometer. The cosine diffuser (Bentham Instruments Ltd. Reading UK) was levelled on the tripod 40-cm high and attached with a fibre-optic cable to the spectrometer. Measurements were corrected for dark-noise and stray light in the UV range (<400 nm) using a protocol detailed by Hartikainen et al. (2018). The spectrometer had been calibrated against a Bentham scanning spectroradiometer for outdoor measurements of solar spectral irradiance, over the wavelength range 290-900 nm. Pre-processing of the data was performed using functions from the R packages Ooacquire and Photobiology in R version 3.4.3 (R Core Team, 2018).

2.4. Optical measurements of *V. vitis-idaea* leaf pigments and photosynthetic capacity

On each measurement date, starting 14-02-2017, then 07-03-2017, and at 2-week intervals subsequently until 08-07-2017, with an additional final measurement date of 08-11-2017, leaf optical properties and photosynthetic capacity were measured. Ten leaves (remaining attached to the plants) were measured *in situ* from each height (upper, middle, lower) on four hummocks. Sun-exposed leaves of equivalent size were measured (or leaves which would have been sun exposed had it not been cloudy on overcast days), all from different shoots of each *V. vitis-idaea* hummock, avoiding the

three distal leaves of a shoot. Lower and middle hummock leaves were, when necessary, excavated from beneath the snow to be measured and subsequently the snow was replaced over these leaves. Leaves that were damp from dew, rain or melting snow were quickly but gently dried with a tissue prior to measurement.

Dualex Scientific^{+} (Force-A, University Paris-Sud, Orsy, France) was used to optically assess the epidermal flavonol concentration (epidermal absorption in the UV-A region at 375 nm) and anthocyanin concentration (absorption at 515 nm), from both the adaxial and abaxial sides of each leaf, as well as the leaf chlorophyll concentration (assessed optically by transmittance in the red at 650 nm vs. near-infra red at 710 nm, Cerovic et al. 2012). We recorded the maximum quantum yield of PS_{II} photochemistry (F_v/F_m; where F_v [variable fluorescence] is the maximal fluorescence, F_m, minus the minimal fluorescence, F_0, of dark-adapted leaves, as defined by Murchie and Lawson, 2013) measured with a mini-PAM (Heinz Walz GmbH, Effeltrich, Germany) following 30 min of dark-adaptation with darkening clips. According to Bokhorst et al. (2010), 30 min in darkness should be sufficient for full dark adaptation of *V. vitis-idaea* leaves year 'round. We also recorded the effective quantum yield of PS_{II} photochemistry (φPS_{II} calculated as $F_q`/F_m`$; where $F_q`$ is the maximal fluorescence, $F_m`$, minus the steady-state fluorescence, $F`$, of leaves in ambient sunlight, as defined by Murchie and Lawson, 2013) of leaves in the sun and shade in the mid-to-late morning. Where possible, the same ten leaves were used for chlorophyll fluorescence and Dualex measurements on any given day, but it was not feasible to measure the same ten leaves from one measurement date to another, so from day to day leaves meeting the above criteria were selected at random.

2.5. Harvest of leaves for trait measurements

On 08-07-2017, leaves of age 29 days and 394 days were harvested. From each age cohort 8-10 leaves were sampled from each of the four hummocks at each of the three heights (2 epidermis × 10 leaves × 3 heights × 4 hummocks). Fresh leaves were placed in zip-lock plastic bags, weighed and digitally photographed against an area reference on the day of sampling, then dried at 40°C for 9 days in a drying oven with forced ventilation before reweighing to obtain their dry weight. Leaf areas were

calculated from the photographs using ImageJ (US National Institute of Health, Bethesda, Maryland, USA: following Wang, 2017) and used to calculate the leaf mass area (LMA mg mm^{-2}). The leaf area covering the optical window of the Dualex Scientific $^{+}$ was 47.7 mm^2 (Area = πr^2, r = 3.90 mm), used with LMA, to calculate per area and per mass values of pigment concentrations on the harvest dates.

2.6. Environmental monitoring of temperature during the experiment

At the time of each measurement of leaf optical properties, the temperature of the leaves of *V. vitis-idaea* at each height on all four hummocks was measured at close range with a high precision infra-red thermometer with special macro optics (LS laser-sight Optris GmbH, Berlin, Germany). Measurements were also recorded at the tree base and at 1 m 50 cm height on the trunks (emissivity *c* 0.98) of the four nearest trees to the hummocks at opposite compass points (North and South at midday) in the sun and shade, and on the snow surface (emissivity of tree trunks and snow *c* 0.98).

Meteorological data for the experimental period were obtained from the SMEAR II ICOS Hyytiälä station through the AVAA data research portal (https://avaa.tdata.fi/). Temperature in the understorey was recorded every minute with a PT100 sensor (Pentronic, Västervik, Sweden) mounted on a mast at 4 m height and soil surface temperature was recorded every minute with a KTY 81-110 sensor (NXP Philips, Amsterdam, Netherlands). Understorey photosynthetically active radiation (PAR) was measured with an array of four Li190SZ PAR sensors (LI-COR, Lincoln, NE) at 0.6m height next to the hummocks. These time series of meteorological data were used to calculate averages over a range of time periods preceding each measurement (1 hour, 1 day, 2 days, and 2 weeks) and the relationship between temperature and PAR over these periods and leaf trait data was plotted. Of these four time integrals the best fit to the trait data was retained.

2.7. Data Analysis

Differences due to hummock height among optically-assessed leaf traits were tested using a two-way ANOVA with hummock height, and leaf age (days) as fixed factors, or a three-way-ANOVA for the responses of flavonols and anthocyanins which were measured in both the adaxial and abaxial epidermis of each leaf (with leaf side as a nested third fixed factor). One measurement date (Leaf Age:

250 days), when only the adaxial epidermis was measured with the Dualex, was excluded from the three-way ANOVA. The equivalent single-factor ANOVA was used to test the effect of hummock height on traits measured only once, e.g. from harvested leaves at the end of the experiment. The four hummocks were the experimental units of replication and height was nested within each hummock. When significance differences were produced by the ANOVA, pairwise differences were identified between the responses across three levels of hummock height on either leaf side on each measurement date (function glht in the package Multicomp). Holm's adjustment was used to account for multiple pairwise comparisons. The relationship of F_v/F_m with temperature and PAR was assessed using linear regression (function lm()). All the analyses were performed using R version 3.4.3 (R core development team, 2018).

3. Results

3.1. Forest cover, snow depth and temperature

The snow depth was *c*. 40 cm at the first winter measurement (14-02-2017), meaning that the upper shoots of hummocks (upper hummocks) were above the snow, the middle of the hummocks were just below the top of the snowpack, and lower shoots of hummocks (lower hummocks) were entirely beneath the snowpack. From 14-02-2017 onwards, the snow depth didn't increase from this maximum and remained above the middle-hummock shoots until 27-03-2017 and above the lower-hummock shoots until snow melt was complete on 10-05-2017.

Soil temperatures under the snow were relatively stable, starting to fluctuate only when snow melt was almost complete and the lower hummock leaves above the snow (Fig. 1; Table 1). From the time series of snow depth and air temperature data, compared with IR-thermometer measurements of snow-pack temperature (Fig. 1), we were able to estimate that the upper-hummock leaves were subject to 111 freeze-thaw cycles, the middle-hummock leaves 86 and lower-hummock leaves 38 such cycles, during the period between New Year and mid-summer 2017 which encompasses full dehardening (Table 1).

3.2. Spectral irradiance at the study site

The solar spectral irradiance was measured on three dates during the winter and spring (14-02-2017, 07-03-2017 and 19-06-2017). On all three dates, the normalised spectra show that PAR was depleted in the under-canopy sunflecks and shade compared with the open field, and the relative contribution of UV radiation was similar in the sunflecks and open sun but enriched in the shade (Figs. 2 and S2, Table S1). The spectral composition of the sunflecks varied with sunfleck size as well as time of year, and the relative contribution to shade by needles and branches also affected the shape of these spectra. The Plant Area Index (PAI) and sunfleck duration on 20-07-2017 were 2.14 ± 0.05 m^2m^{-2} and 169.6 ± 11.27 min day^{-1} respectively, and was similar among the four hummocks (Table S2).

The reflectance (up to 90%) and transmittance of sunlight by the snowpack modifies amount and composition of radiation received by leaves above and below the snowpack at different hummocks heights. We found the snowpack to transmit about 10% of incident PAR irradiance to a depth of 10 cm, and a similar or slightly higher percentage of UV radiation to reach the same depth (Robson unpublished data) in line with comparable measurements elsewhere (Richardson and Salisbury, 1977; Gorton and Vogelmann, 2003; Perovich 2007).

3.3. Seasonal changes in leaf pigments at different heights on the *V. vitis-idaea* hummocks

Leaf flavonoid concentration (i.e. both flavonols and anthocyanins), measured optically and presented as an index of absorption on a per leaf-area basis, was most similar directly after leaf unfolding both among hummocks and between leaf sides. As the leaves matured, their flavonol concentration increased in the adaxial epidermis and initially decreased in the abaxial epidermis between the first and second measurement of new leaves during the summer (Leaf Age 10-29 days, Fig. 3A). During this period, UV-screening by flavonols was greater in the upper-, and middle-, hummock leaves than the lower leaves. Accumulation of flavonols continued until the start of winter in the adaxial-, and to a lesser extent the abaxial-, epidermis, and only started to definitely decline with age following the spring and summer of their second year (Leaf Age 271 days; Fig. 3A). Leaves of

278 *V. vitis-idaea* can remain productive for 5 years or more, but after their first growing season maximum
279 photosynthesis declines sharply (Karlsson, 1985).

280 Adaxial epidermal flavonol concentration in *V. vitis-idaea* leaves transiently decreased during
281 winter in lower- and mid-hummock leaves when they were beneath the snowpack, but started to
282 increase again from 24-03-2017 (Leaf Age 288 days) coinciding with temperatures rising above
283 freezing. In sequence, the upper, then middle, then lower hummock leaves' adaxial flavonol
284 concentration increased around the time that the middle hummocks leaves emerged from the
285 snowpack (Fig. 3A, from 27-03-2017, Leaf Age 291 days). This phase of flavonol accumulation
286 continued until the end of the snow melt period (16-05-2017 Leaf Age 341 days), after which flavonol
287 concentration was stable and eventually started to decline from 19-06-2017 Leaf Age 375 days (Fig.
288 3A). Flavonol accumulation in the adaxial epidermis during spring was greater than that in the abaxial
289 epidermis, leading the adaxial-to-abaxial flavonol ratio to increase until 08-07-2017, Leaf Age 394
290 days, but it returned to the equivalent of pre-spring ratios later in the year (08-11-2017, Leaf Age 517;
291 Figs. 3A and S3A).

292 Leaf anthocyanin concentration is typically highest in young leaves, and higher in the abaxial
293 than the adaxial epidermis, declining once leaves are mature (Landi et al. 2015): our results were
294 consistent with this pattern until late winter, when anthocyanin accumulation rebounded (07-03-
295 2017, Leaf Age 271 days, Fig. 3B). Initially, there were no differences in anthocyanin concentration
296 with respect to height on the hummocks, and this remained the case until 24-03-2017 (Leaf Age 313
297 days) after the middle-hummock had emerged but while the lower hummock was still beneath the
298 snow pack. During this period (24-03-2017 to 18-04-2017), adaxial epidermal anthocyanins continued
299 to accumulate in the upper hummock leaves (Fig. 3B) and the difference in adaxial epidermal
300 anthocyanin concentration with height was maintained, with higher values in upper hummock leaves
301 compared with the others (Fig. 3B). In the abaxial epidermis, a corresponding difference with height
302 occurred later during the 6-week spring dehardening period (19-04-2017 to 30-05-2017: Leaf Age 313-

355 days), persisting until abaxial anthocyanin concentration was maximal in the upper and mid hummock leaves between (17/30-05-2017, Leaf Age 341-355 days). By this time, the abaxial epidermal anthocyanins had already started to decline in the lower-hummock (Fig. 3B). By mid-summer, both adaxial and abaxial epidermal anthocyanins had converged to similar values across the three height classes (19-06-2017: Leaf Age 375) (Fig. 3B). The ratios of adaxial-to-abaxial epidermal anthocyanins all declined throughout the spring, but differed among the three heights, being highest in the upper-hummock leaves and lowest in the lower-hummock leaves (Fig. S3B).

Chlorophyll concentration per leaf area increased in young leaves until 14-02-2017 (Leaf Age 250 days), by which time it was lower in the lower-hummock leaves than the middle-and-upper-hummock leaves (Fig. 3C). This difference persisted until the upper and middle hummocks were above the snowpack but the lower hummock beneath the snowpack (18-04-2017, Leaf Age 313 days), at which time chlorophyll concentration of the lower-hummock leaves increased beyond that of the exposed upper and middle hummock leaves where it declined (Fig. 3C). Lower-hummock leaves retained the highest chlorophyll concentration from then until midsummer by which time chlorophyll values at the three heights converged again (19-06-2017, Leaf Age 394 days) (Fig. 3C).

The ratio leaf flavonols vs. anthocyanins (sum of both epidermis) was higher in the upper hummocks than the lower hummocks through the winter (Fig. 4A). In the adaxial epidermis, the rate of flavonol accumulation was faster than that of anthocyanins during the dehardening period so this ratio rose, particularly in the lower hummock leaves (due to increasing flavonol accumulation as described above - Fig 4A). There was negative relationship between flavonols and anthocyanins in the adaxial epidermis when data are plotted for all leaves and all dates (Fig. 4B). This relationship may be confounded by leaf age but nevertheless persisted for the adaxial epidermis when the relationships were calculated separately for each measurement date (Table S5).

3.4. Seasonal changes in photosynthetic yield of PS_{II} in leaves at different heights on the *V. vitis-idaea* hummocks

The F_v/F_m of leaves across the three heights on the hummocks attained their maximal values of about 0.82 on reaching maturity on their second measurement following unfurling (09-07-2017, Leaf Age 30 days) and F_v/F_m remained high prior to snow cover in the winter (Fig. 5A). During the winter and spring, F_v/F_m was depressed most in the upper hummocks, then middle hummocks and least depressed in the lower hummocks (Fig. 5A). This ranking of F_v/F_m according to height on the hummocks was retained throughout the spring dehardening period (until 30-05-2017: Leaf Age 355 days), but by midsummer one-year old leaves at all three heights had attained similar maximal F_v/F_m again (19-06-2017, Leaf Age 375 days) (Fig. 5A).

The φPS_{II}, measured *in situ* under ambient sunlight, varied with the solar irradiance, i.e. on clear-sky measurement days φPS_{II} was very low during the dehardening period (Fig. 5B). At sub-zero temperatures (e.g. -5.5 °C on 07-03-2017, Leaf Age 313 days) with clear-sky irradiance during early spring, the φPS_{II} was lowest and approached zero. Though bear in mind that it was necessary to remove the snow from covered leaves during winter to take the measurement, exposing them briefly to higher PAR than they would otherwise have received and thus probably caused greater photoinhibition than when under the snow. Nevertheless, in early spring on days 83 and 96 (leaf age 288 and 301 days), φPS_{II} of almost 0.2 was reached in the lower hummock leaves (Fig. 5B). Both measures of photosynthetic capacity (F_v/F_m and φPS_{II}) responded to day-to-day differences in environmental conditions, as evident from differences between F_v/F_m values from consecutive daily measurements made on three occasions during the spring dehardening period (Fig. 5B, Leaf Age 313-314, 327-328, 341-342 days).

3.5. The relationship between *V. vitis-idaea* photosynthetic yield, leaf flavonoids and environmental conditions

We tested the relationship between temperature and F_v/F_m for leaves from the upper, middle, and lower hummocks, identifying their position relative to the snowpack prior to each F_v/F_m

measurement. The air temperature (T_{aw}) or soil temperature (T_{sw}) at the site were positively correlated with the average F_v/F_m of leaves above or below the snowpack respectively, and in order to make a direct comparison with other studies of *V. vitis-idaea* (Bokhorst et al. 2010; Saarinen et al. 2016), we tested a range of different time periods to find the closest relationship. Of these time periods, the temperature over the 2 weeks prior to each F_v/F_m measurement gave the best fit; for leaves above the snowpack vs. T_{aw} (adj. R^2=0.91, Table S4), and leaves below the snowpack vs. T_{sw} (adj. R^2 = 0.81, Table S4). These relationships with temperature were stronger than F_v/F_m vs. PAR on the day of measurement for leaves above the snowpack (adj. R^2 = 0.74, Table S4).

Individual leaf measurements of F_v/F_m were also positively correlated with leaf temperature (Fig. 6A) and PAR incident on the leaf (Fig. 6B) at the time of measurements, and likewise with adaxial-epidermal flavonol concentration on the day of measurement (Fig. 6C). However, these relationships were strongly conditioned by measurement day and held only during the period from 24-03-2017 to 17-05-2017: Leaf Age 281-342 days (Fig. 6). The relationships broke down once spring recovery of F_v/F_m was almost complete (30-05-2017 Leaf Age 355 days – not shown) and in winter when leaf temperatures were consistently around 0°C. During dehardening, all three relationships were strongest in the upper hummocks and remained strong in the middle hummocks, where F_v/F_m recovery coincided with an increase in adaxial epidermal flavonols during this time period, but were but much less strong in the lower hummocks (Fig. 6C). The same relationship was not evident for anthocyanins in either epidermis (not shown).

4. Discussion

4.1 Consistent pattern of increasing flavonoids with hummock height throughout the year

Differences in exposure to harsh weather conditions among adjacent plants or shoots may be particularly pronounced in the understorey of open canopies typical of the boreal forest which unevenly intercept solar radiation (both incoming and reflected or emitted radiation) and snow (Hedstrom and Pomeroy, 1998). The undulating topography created by hummock-forming plants, adds to the heterogeneity of irradiance, temperature, and snow cover (Essery et al. 2008; Pomeroy et

al. 2002; Sturm et al. 2001). Accordingly, a large proportion of their leaves can remain above the snowpack over winter where they are subject to greater fluctuations in temperature and irradiance than those beneath the snowpack. In our study, this was the case for the upper leaves of *V. vitis-idaea* hummocks, which accumulated more epidermal UV-screening (attributable to flavonols) than lower hummock leaves. Using a Dualex to optically detect UV-screening allowed us to repeatedly and non-destructively follow changes over time on the same plant but not to gather biochemical information on flavonoid composition.

The results of studies based on optical properties of the leaf epidermis *in vivo* and measurements of whole-leaf extracts can be difficult to reconcile because the two methods are not equivalent. In *V. vitis-idaea*, flavonoids can be found in the trichomes and bound to the epidermal cell walls (Semerdjieva et al. 2003a, 2003b) where they will affect UV-screening, whereas they are spread throughout the leaf including chloroplasts in the mesophyll and cuticular waxes in species like *V. myrillus* (Semerdjieva et al. 2003b). Despite a certain lack of equivalency, it is worth noting that the relative differences we found in UV-screening within *V. vitis-idaea* hummocks, among leaves differing only in the time spent above and below the snowpack (Fig. 1), were larger than those produced in the same species by a 25% UV supplementation (Semerdjieva et al. 2003b). A strong negative correlation between accumulation of leaf flavonoids and increasing temperature can obscure the effects of UV-B radiation (Coffey et al. 2017), and consequently, the differences in temperature across hummock height related to differences in seasonal snow cover may partially explain the vertical gradient in flavonols accumulation we report. It has not yet been elucidated whether both temperature and UV-B radiation may regulate flavonoid accumulation via the uvr8 pathway, but this would constitute an interesting future avenue of research.

The response of *V. vitis-idaea* leaves to UV radiation at high latitudes has already been extensively studied in the context of northern-hemisphere stratospheric ozone depletion in the late 1990s and 2000s (e.g. Johansson et al. 1995; Phoenix et al. 2001; Semerdjieva et al. 2003a). The

403 leaves of evergreen species emerge from snow cover in spring when seasonal UV-B exposure can be
404 periodically high, but a meta-analysis generally found evergreen species like *V. vitis-idaea* to be
405 resilient against such seasonal increases in UV radiation (Newsham and Robinson, 2009). The
406 modest flavonoid induction caused by increased UV-B radiation could help protect from
407 photodamage to PS_{II} and improve antioxidant capacity during dehardening at the start of the
408 growing season when high irradiance and cold temperatures in unison exacerbate photoinhibition
409 (Takahashi and Badger 2011).

410 Most studies from the 1990s-early-2000s have presented these data on a per mass basis
411 which is not as easily interpretable as leaf-area based measures in terms of screening. Differences in
412 LMA among the one-year old leaves in our experiment caused the effect of hummock height on
413 flavonols and anthocyanins per-leaf-area to be reduced when considered on a per-mass basis (Table
414 2). Likewise, the effect of UV-B radiation on flavonol accumulation is sometimes smaller on a per-
415 leaf-mass than per-leaf-area basis in understorey shrubs, because UV-B exposure causes mature
416 leaves to be thicker and tougher (Newsham and Robinson, 2009, Semerdjieva et al. 2003a).

417 The increase in the ratio of adaxial to abaxial epidermal flavonols in the lower hummock
418 leaves (Fig. S3A) during snow melt suggests that accumulation of adaxial flavonols could aid in
419 photoprotection in addition to amelioration of cold temperature stress as antioxidants (Chalker-
420 Scott and Scott, 2004). Comparison of the UV screening on either side of a leaf show this
421 relationship to be highly species specific and environmentally dependent: sometimes UV screening
422 by the adaxial and abaxial epidermises are strongly positivity correlated (Bartlod et al. 2007, Siipola
423 et al. 2014) sometimes this relationship is weak (Kolb and Pfündel 2005, Morales et al. 2011), as was
424 the case in our study. The accumulation of epidermal flavonoids is commonly attributed to exposure
425 to UV-B radiation, or high irradiance of UV-A radiation and blue light (Barnes et al., 2017), although
426 flavonoid accumulation is also known to increase in response to cold temperatures (Agati et al,
427 2013). Temporal differences in flavonoid (both flavonol and anthocyanin) accumulation and their

localization in the planophile leaves of *V. vitis-idaea* might reflect differences in the allocation of resources based on differences between the adaxial and abaxial epidermis in their relative exposure to cold and high irradiance.

Over the whole dataset, there was an inverse correlation between the anthocyanin and flavonol concentrations in the adaxial epidermis, even though during spring dehardening both tended to increase (Fig. 4). A similar pattern showing a trade-off between these two phenolic compounds was reported in *Arabidopsis thaliana* under controlled conditions, where the ratio of flavonoids to anthocyanins was higher when they received either blue light or UV-A radiation (Brelsford et al, 2018). Anthocyanins are typically found in greater amounts in young leaves and have a specific role as antioxidants involved in cold and high-light tolerance (Havaux and Kloppstech, 2001; Landi et al. 2015). Although anthocyanins only infrequently respond to elevated UV-B radiation (Searles et al. 2001), both flavonols and anthocyanins are phenolic compounds of the flavonoid pathway and it is possible that regulation of this pathway shifts during spring to emphasize the photoprotective function of flavonols driven by seasonally-increasing irradiance of blue light and UV radiation at the expense of anthocyanins whose antioxidant function would be more important during winter (Agati et al. 2013).

4.2 Prolonged snow cover accelerates recovery of photosynthetic capacity during spring dehardening

The timing of spring dehardening in our study was better correlated with temperature than irradiance, and the relationship with temperature was maintained over 2 weeks prior to measurement (Fig 6), in agreement with Karlsson (1985). Several studies have found a consistent relationship between the ambient temperature and F_v/F_m of *V. vitis-idaea* leaves across a range of latitudes (Bokhorst et al. 2010; Saarinen et al. 2016; Table S6 and S7) and in *Pinus sylvestris* at our Hyytiälä site (Porcar-Castell et al. 2011). Saarinen et al. (2016) report that F_v/F_m in leaves of *V. vitis-idaea* plants was depressed by snow removal or even when covered by only a thin layer of snow. In our study, upper-hummock leaves had the lowest F_v/F_m and mid-hummock leaves, which were also only thinly

454 snow-covered, had lower F_v/F_m than lower-hummock leaves. Prior to their emergence from under the
455 snow, the F_v/F_m of lower-hummock leaves was already increasing. It is conceivable this recovery was
456 aided by the low to moderate irradiance that penetrates the upper cm's of the snowpack, potentially
457 activating photoprotective mechanisms during snowmelt (Richardson and Salisbury, 1977). This could
458 partially explain why the difference in F_v/F_m was maintained between leaves at different heights on
459 the hummocks for several weeks following snow melt rather than the lower leaves exhibiting a cold-
460 shock response upon exposure.

461 Large fluctuations in temperature and sustained winter warming can both prematurely activate
462 dehardening creating the potential to bring forward the growing season but also increasing the risk of
463 damage if cold winter temperatures return (Bokhorst et al. 2008). Photoinhibition of exposed shoots
464 was high in our study, and leaf chlorophyll concentration dropped in the upper and middle hummock
465 leaves during the spring dehardening period but remained stable in the lower hummock leaves
466 (surpassing that in the upper hummocks) following emergence from under the snowpack. The
467 regulation of leaf chlorophyll may be a means of acclimation, to reduce photon capture under high
468 irradiance to balance the limited carboxylation capacity at low temperature in the upper and middle
469 hummocks when photoprotective mechanisms were surpassed.

470 Winter- and spring-time depression of F_v/F_m and φPS_{II} was largely attributable to a decrease in
471 maximal fluorescence (F_m and F_m` respectively) rather than an increase in $F_0$ or $F_0$` (Fig. S5). This
472 suggests that the reduction in photosynthetic capacity is the result of decreased light harvesting by
473 PS_{II} lowering the F_m and F_m`. Such a result has previously been attributed to smaller antenna size or
474 sustained NPQ associated with decreased epoxidation of xanthophylls or PS_{II} reaction centre
475 quenching under cold temperatures (Ivanov et al. 2008), rather than damage or disassembly of the
476 PS_{II} LHC_{II} core subunit super-complexes which would be reflected in higher F_0 or F_0` (Öquist and Huner,
477 2003). The positive correlation between epidermal flavonols and F_v/F_m found in the upper and middle,
478 but not lower, hummock leaves during dehardening suggests that these leaves are acclimating to the

more-exposed conditions. This is consistent with some mechanistic studies which report flavonoids to determine of cold acclimation and frost tolerance of *Arabidopsis thaliana* (Havaux and Kloppstech, 2001; Shulz et al. 2016). However, knowledge of the contribution of xanthophyll cycle pigments at different hummock heights, which was not quantified in this study, would allow us to better describe this response (Adams et al. 2004).

4.3 Ecological consequences of changing snow cover for *V. vitis-idaea*

In our study in central Finland, all leaves achieved full functionality following dehardening. Likewise, Saarinen et al. (2016), found that exposed leaves of *V. vitis-idaea*, subjected to experimental removal of the snow pack during winter, fully recovered PS_{II} capacity during the spring in southern Finland, abet later than those from which snow was not removed. This result is consistent with other Arctic shrub species, e.g. *Rhododendron ferrugineum* L, whose leaves above the snow in winter have transiently reduced F_v/F_m compared with those that are covered by snow (Neuner et al. 1999). These findings suggest that *V. vitis-idaea* could benefit from the expected warmer winters and earlier snow melt in Finland, and much of the rest of its range (Rasmus et al. 2004 Henry 2008; Jylhä et al. 2008), by dehardening earlier and contributing more in the future to photosynthetic carbon gain in the understorey (Gehrmann et al., 2018; Larsen et al. 2007). However, this does not exclude the possibility that environmental changes involving reduced duration or size of snowpack can also be detrimental to understorey plants. Further north than our study, across a range of habitats in the Arctic where winters are colder than in central Finland, a reduced snow pack can cause sufficient cold stress to damage the leaves and shoots of shrubs including *V. vitis-idaea* (Preece and Phoenix 2013). Reduced snow cover can also lead to colder soils and prolonged freezing temperatures which can be a problem over a wider geographical range across Europe causing root mortality and affecting biogeochemical cycling (Groffman et al. 2001; Helama et al. 2011).

The link between increasing temperature and irradiance, and F_v/F_m recovery in *V. vitis-idaea* during dehardening reinforces established knowledge of this and other species (Ensminger et al. 2004; Havaux and Kloppstech 2001; Muller et al. 2005; Neuner et al. 1999; Porcar-Castell et al. 2008;

505 Saarinen et al. 2016). At our site in central Finland, winter temperatures are relatively mild (-7.37 ±
506 1.15 °C; 10-year mean ± 1 SD January temperature, 2008-2018), and are expected to increase by 4-
507 11 °C by the end of the century (Jylhä et al. 2008; Finland's Seventh National Communication under
508 the United Nations Framework Convention on Climate Change 2017). Winter warming is forecast to
509 increase productivity at the stand level (Porcar-Castell et al. 2011). However, the expected 30-40%
510 reduction in snow cover (Jylhä et al. 2008; Finland's Seventh National Communication under the
511 United Nations Framework Convention on Climate Change 2017) may lead to increased soil freezing
512 or freeze thaw cycles which may partially negate the increase in productivity (Taulavuori et al. 2011).
513 New estimates of ecosystem carbon assimilation aim to include the contribution of understorey
514 vegetation in addition to that by tree species (Nikopensius et al. 2015, Starr and Oberbauer, 2003).
515 To estimate understorey photosynthesis during spring a better understanding of how it is affected
516 by uneven snow cover under patchy canopies will be needed (Blume-Werry et al. 2016; Kreyling et
517 al. 2012).

518 ## 5. Conclusions (preferably 10-15 lines & no references)

519 In our study, there was a positive correlative relationship during the winter and spring
520 between UV-screening by epidermal flavonols and F_v/F_m in leaves that were largely above the
521 snowpack. More flavonols and anthocyanins accumulated in these upper-hummock leaves
522 compared to leaves below the snowpack. The cross-protection provided by epidermal flavonols to
523 cold and high light is likely to be regulated by solar radiation and temperature, but further research
524 is needed to disentangle the roles of these two cues. Recent evidence that leaves can rapidly adjust
525 their UV-screening to acclimate to light conditions (Barnes et al. 2017) suggests that a high-degree of
526 fine tuning of these responses is possible, and our finding that flavonoids (flavonols and
527 anthocyanins) accumulate in the upper hummocks even in cold conditions when metabolic activity is
528 low supports this assertion. Leaves with a higher index of epidermal flavonols tended to have a
529 lower epidermal anthocyanin index, and a compositional shift in this ratio towards flavonols during
530 spring suggests fine-tuning of acclimation in response to increasing irradiance and a less cold

temperatures. Finally, on hummocks at our site in central Finland, leaves that remained under snow cover longer surpassed exposed leaves in their F_v/F_m directly following snowmelt despite having lower epidermal flavonols and anthocyanins but all leaves eventually reached a similar maximum photosynthetic capacity.

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Tables

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784 2017 to 18-05-2017) when the temperature is above freezing but leaves have not yet recovered to
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786

Online Supplemental Material

Table S1: Spectral photon irradiance at the *V. vitis-idaea* hummocks. Spectral integrals and photo ratios calculated from spectral irradiance (Fig S2) measured in shade and sunflecks on three different dates (Feb, 14-02-2017; Mar 07-03-2017; Jun 19-06-17). The mean ± 1SE for the four hummocks are given plus the % decrease compared with adjacent open full sun measurements.

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Table S3: Summaries of three and two-way ANOVA tables for the effects of hummock height, leaf side, leaf age, and their interactions on leaf pigments (Figs 3 and 4), chlorophyll fluorescence (Fig. 5), quenching (Figs S4 and S5), Leaf area and mass-based pigment values (Table 2) (df = degrees of freedom)

Table S4: Relationship between the maximum quantum yield of PS_{II} (F_v/F_m) and air and soil temperature tested for leaves at each hummock height. Linear regressions for leaves above and below the snowpack were tested against air and soil temperature respectively (2 weeks only). The mean of F_v/F_m over 4 hummocks on each of 6 measurement dates during the period of spring dehardening was used (leaf age 228-375, 24-03-2017 to 19-06-2017).

Table S5: Relationship between epidermal anthocyanins and flavonols for adaxial and abaxial epidermis separately. Each measurement date is tested separately for leaves at each hummock height.

Table S6: Relationships calculated from Saarinen et al, (2010) between air and soil temperatures and F_v/F_m of *V. vitis-idaea* leaves at the time of measurement on date 2009-03-23.

Table S7: Relationships calculated from Bokhorst et al, (2010) between air and soil temperatures and F_v/F_m of *V. vitis-idaea* leaves at the time of measurement in 2008 and 2009.

Figure S1: Grey scaled images and binary images with/without Floyd-Steinberg dithering created with IrfanView 4.44 for all the original hemispherical photos on the hummocks sampled at the measuring site on 20-07-2017.

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Fig. S3: Ratio of adaxial to abaxial epidermal (A) flavonol and (B) anthocyanin indices, measured by Dualex. Ratios calculated from the data presented in Fig. 3, over the period from leaf emergence until leaves of *V. vitis-idaea* were one-and-a-half years old. Mean $\pm$ 1 SE of the 4 hummocks (n=4) are plotted at 3 hummock heights, and the period under the snowpack marked with vertical lines. * indicates a significant difference with height on the adjacent date, ANOVA summaries in Table S3.

Fig. S4: The parameters F_m and F_0 (A & B) are used to calculate F_v/F_m, the maximum quantum yield of photosystem II photochemistry (Fig 5A), and F_m` and F (C & D) are used to calculate φPSII the effective quantum yield of photosystem II photochemistry (Fig 5B). The *ETR* (E), electron transfer rate is calculated from φPSII and PAR incident on the leaf (F). The period covers leaf emergence until leaves of *V. vitis-idaea* were one-and-a-half years old is given. Mean $\pm$ 1 SE of the 4 hummocks (n=4) are plotted, and the period under the snowpack marked with vertical lines. Quenching coefficients calculated from these data in Fig. S5.

Fig. S4: A. The photochemical ($_qP$) and non-photochemical quenching (NPQ) and fraction of PSII centers that are 'open' ($_qL$) calculated from chlorophyll fluorescence measurements, defined according to Baker, 2008 (Chlorophyll Fluorescence: A Probe of Photosynthesis In Vivo, Annu. Rev. Plant Biol. 2008. 59:89–113 doi:10.1146/annurev.arplant.59.032607.092759). Time series over the

834 period from leaf emergence until leaves of *V. vitis-idaea* were one-and-a-half years old. Mean ± 1 SE

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838 of times that the temperature passed above and returned below freezing during this period. Tree trunk temperatures were recorded at breast height on the
839 completed shaded north side of the tree during winter and spring.

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Date of Measurement	DOY	Leaf Age Days	Max-Air Temperature °C	Min-Air Temperature °C	Max-Soil Temperature °C	Min-Soil Temperature °C	Max-PAR µmol $m^{-1}s^{-1}$	Freeze-thaw cycles	Snow Temperature °C	Tree Trunk Temperature (North)°C
14-02-2017	45	250	5.3	-1.3	0.5	0.3	112.9	34	--	--
07-03-2017	66	271	-0.4	-10.4	0.0	-1.2	289.5	8	-7.9	-6.6
24-03-2017	83	288	1.8	-4.4	0.4	0.2	474.7	14	-10.0	-4.2
06-04-2017	96	301	8.6	-0.3	0.5	0.4	764.4	15	-3.5	0.8
18-04-2017	108	313	3.7	-7.3	0.3	-0.2	677.0	--	-2.6	-1.4
19-04-2017	109	314	5.5	-5.9	0.4	-0.1	519.8	14	-2.7	2.2
02-05-2017	122	327	12.2	0.2	0.6	-0.4	828.9	--	-3.0	8.7
03-05-2017	123	328	10.9	0.6	0.8	0.5	868.8	17	-2.8	8.1
16-05-2017	136	341	13.5	-2.7	3.4	1.1	919.6	--	--	7.7
17-05-2017	137	342	10.4	1.6	3.7	2.1	271.1	16	--	6.6
30-05-2017	150	355	13.6	0.1	7.6	4.7	290.0	--	--	9.8
19-06-2017	170	375	20.9	9.7	11.9	9.9	932.3	3	--	--
08-07-2017	189	394/29	20.3	7.2	12.7	9.2	836.8	1	--	--
09-07-2017	190	395/30	21.9	9.9	13.9	10.3	899.6	--	--	--
08-11-2017	312	517/152	5.2	2.7	4.3	0.4	51.7	34	--	--

842

843 Table 2: Comparison of leaf pigment measurements and leaf morphological traits on per area and per mass basis. Final harvest of one-year old leaves (leaf
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845 middle hummocks from lower hummock values are given in parentheses. ANOVA results given in Table S5.

Leaf Trait	**Young leaves (Leaf Age 29 days old)**			**One-year old leaves (Leaf Age 394 days old)**		
	Lower Hummock	**Middle Hummock**	**Upper Hummock**	**Lower Hummock**	**Middle Hummock**	**Upper Hummock**
Morphological Trait						
Leaf Area (mm^2)	240 ± 9	240 ± 15 (0%)	246 ± 12.3 (+3%)	174 ± 11	206 ± 27 (+18%)	188 ± 20.6 (+8%)
Leaf Dry Matter Content (mg)	14.5 ± 1.9	13.6 ± 1.5 (-6%)	13.7 ± 1.6 (-5%)	28.9 ± 2.4	35.8 ± 4.8 (+24%)	34.9 ± 3.9 (+21%)
Leaf Mass Area (mg mm^{-2})	0.060 ± 0.006	0.056 ±0.003 (-6%)	0.055 ±0.005 (-8%)	0.166 ± 0.005	0.174 ± 0.01(+5%)	0.187 ± 0.01(+12%)
Dualex (per area)						
Chlorophyll index per leaf area (Dualex A mm^{-2})	78.6 ± 5.8	74.6 ± 6.4 (-5%)	77.8 ± 4.8 (-1%)	122.6 ± 5.6	136.8 ± 23.4 (+12%)	121.7 ± 17 (-1%)
Epidermal flavonol index per leaf area (Dualex A mm^{-2})	5.6 ± 0.7	6.8 ± 0.4 (+22%)	8.0 ± 0.7 (+43%)	5.7 ± 0.1	7.2 ± 0.9 (+25%)	6.8 ± 0.9 (+19%)
Epidermal anthocyanin index per leaf area (Dualex A mm^{-2})	0.89 ± 0.01	1.03 ± 0.07 (+16%)	1.07 ± 0.08 (+20%)	0.26 ± 0.03	0.36 ± 0.03 (+39%)	0.34 ± 0.02 (+29%)
Dualex (per mass)						
Chlorophyll index per leaf mass (Dualex A mg^{-2})	1346 ± 162	1322 ± 52 (-2%)	1445 ± 173 (+7%)	739 ± 14	798 ± 154 (+8%)	657 ± 99 (-11%)
Epidermal flavonol index per leaf mass (Dualex A mg^{-2})	93 ± 6	120 ± 4 (+30%)	146 ± 13 (+57%)	35 ± 1	42 ± 6 (+20%)	37 ± 5 (+6%)
Epidermal anthocyanin index per leaf mass (Dualex A mg^{-2})	15.2 ± 1.3	18.3 ± 0.6 (+20%)	19.4 ± 0.3 (+27%)	1.6 ± 0.2	2.1 ± 0.3 (+34%)	1.8 ± 0.1 (+15%)

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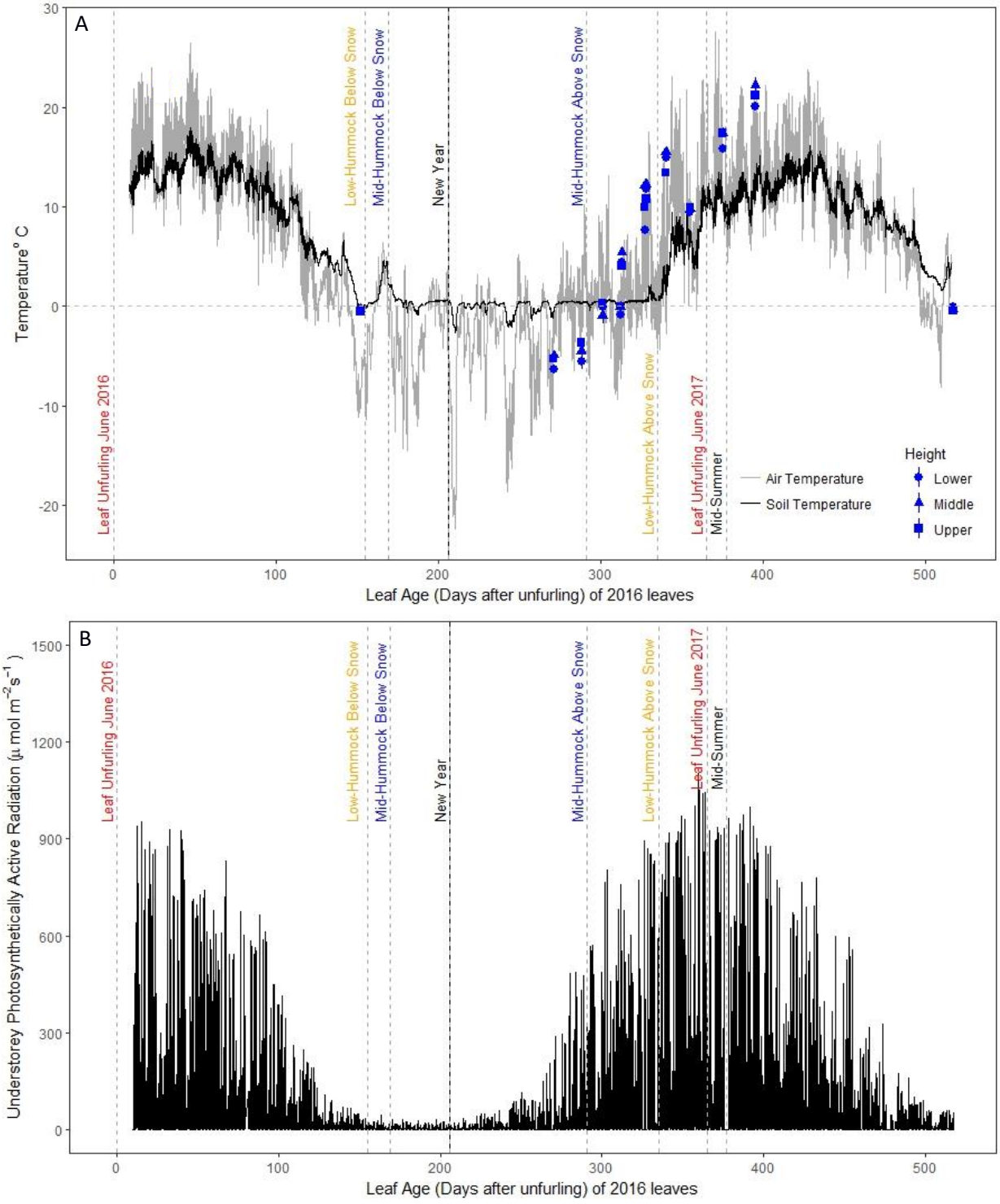

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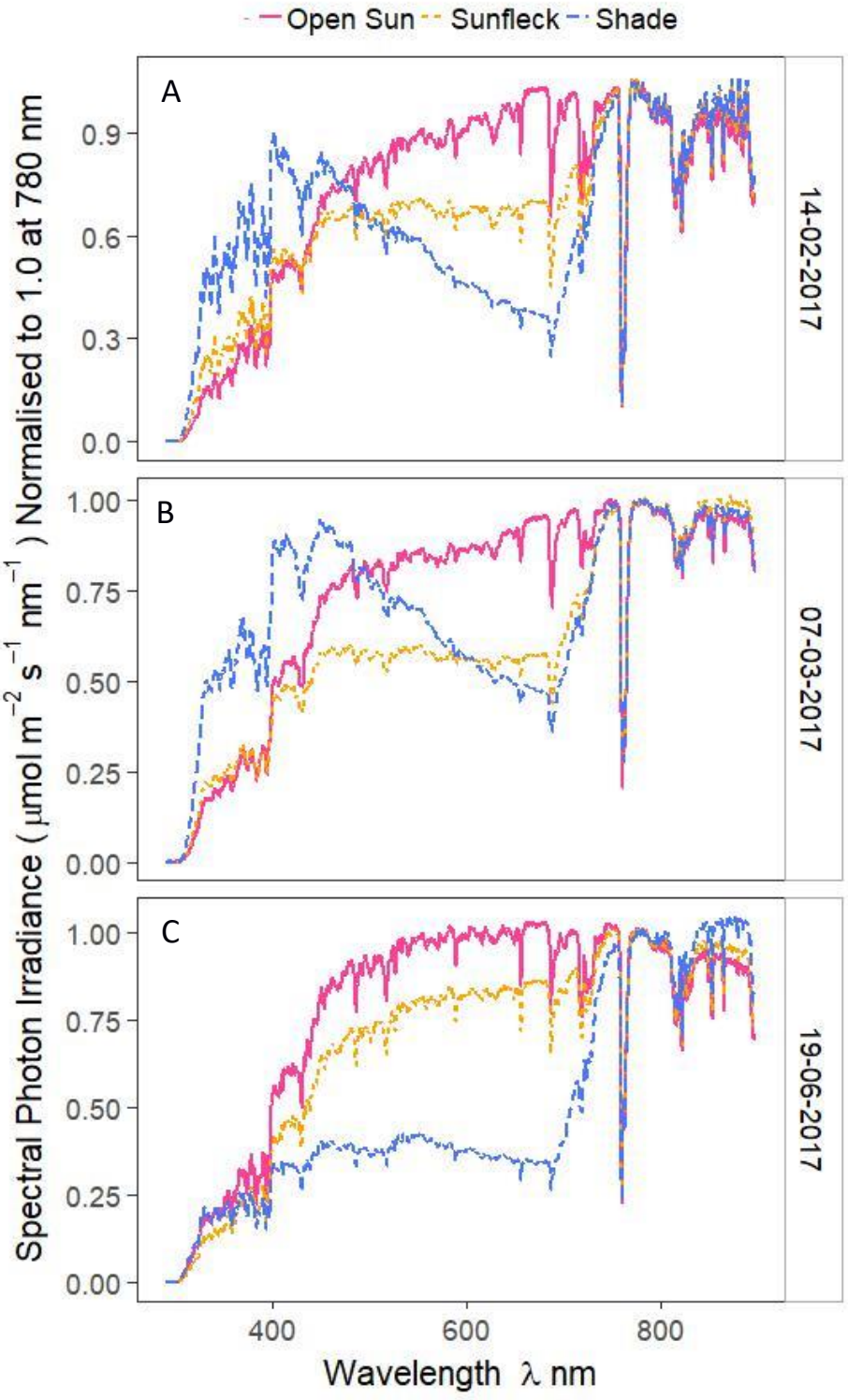

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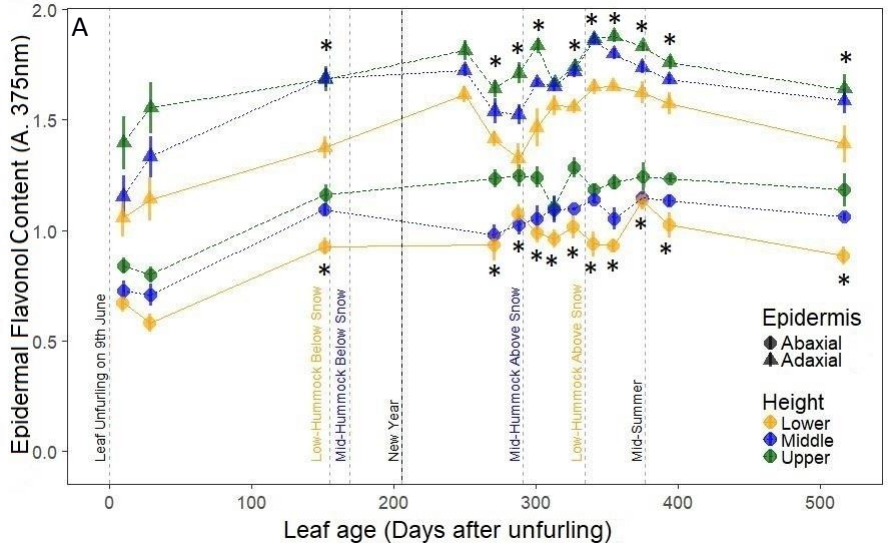

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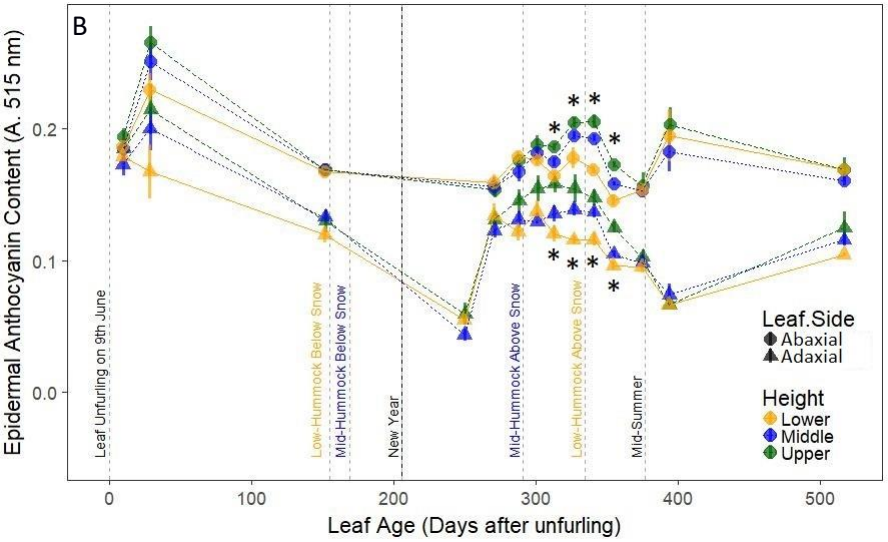

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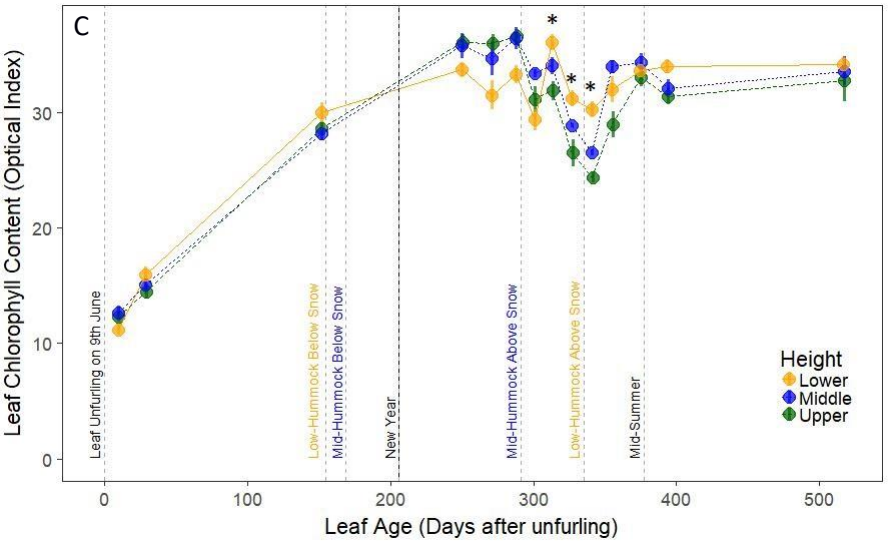

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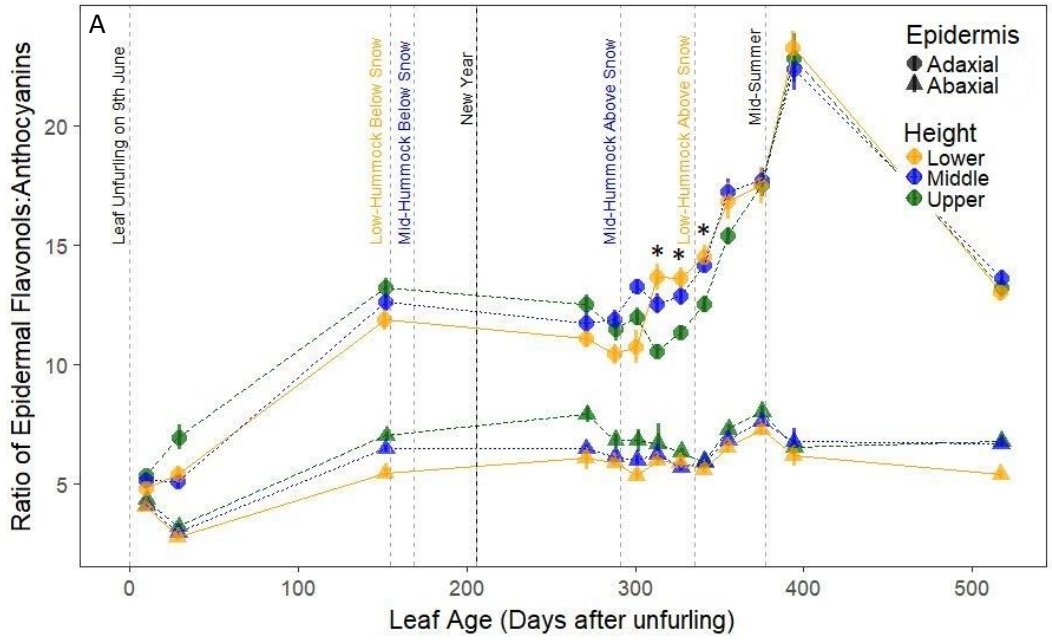

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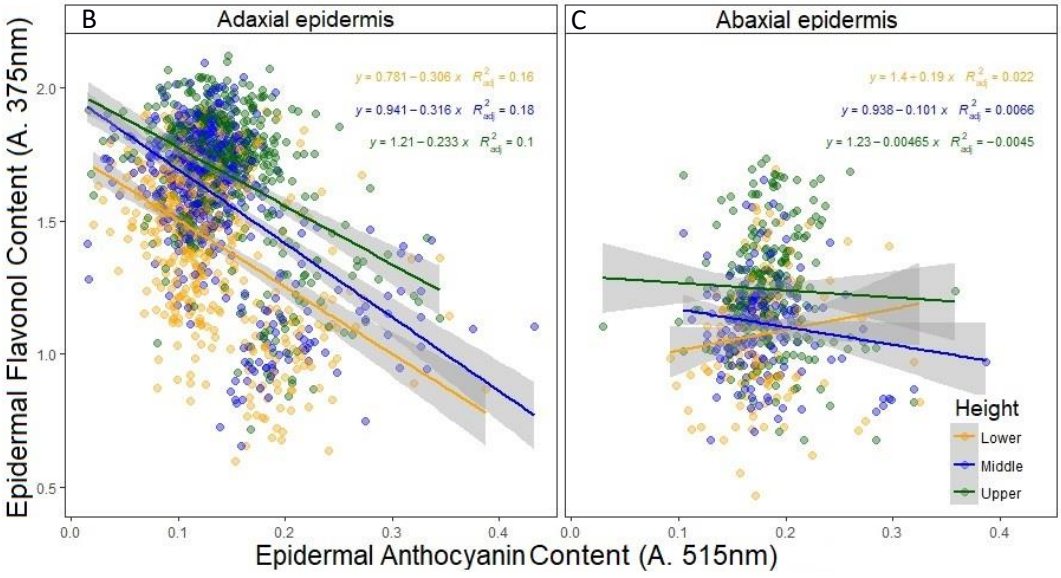

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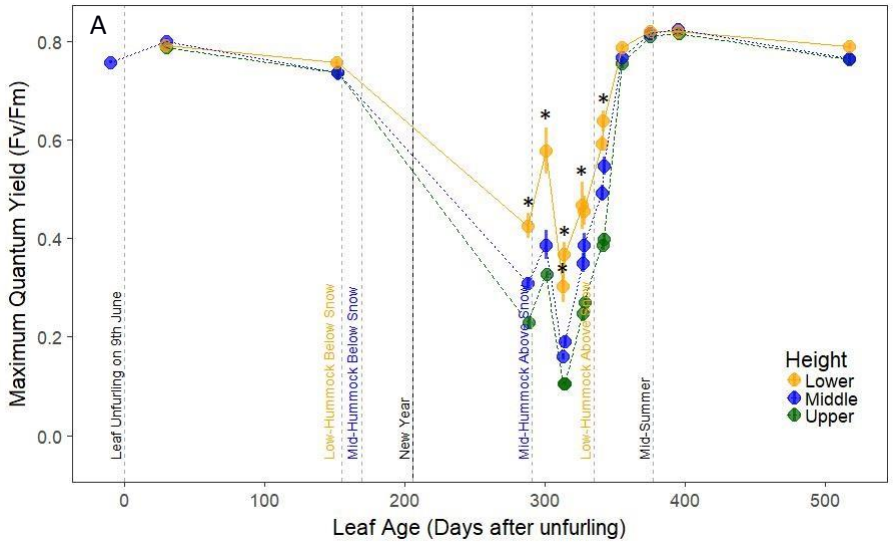

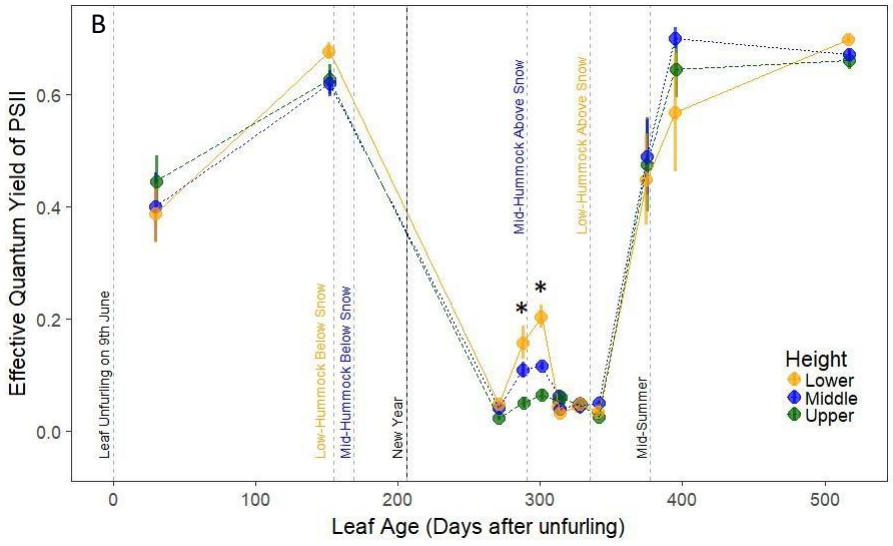

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